Serial Number: 09/988384 Filing Date: November 19, 2001

Title: PROMOTER FOR METHYMYCIN AND PIKROMYCIN

In the Claims

Please amend the claims as follows:

- 1-28. (Canceled)
- 29. (Withdrawn) A recombinant bacterial host cell comprising a deletion of the thioesterase domain of *pikAIV* gene.
- 30. (Withdrawn) The recombinant host cell of claim 29 further comprising a deletion in the *pikAV* gene.
- 31. (Currently Amended) An isolated and purified DNA molecule eomprising consisting of a DNA segment comprising a pikA promoter having at least 90% nucleic acid sequence identity to a DNA fragment corresponding to a nucleotide sequence between an EcoRV site and EcoRI site in SEQ ID NO:5.
- 32. (Currently Amended) An expression cassette comprising a *pikA* <u>DNA segment having a</u> promoter operably linked to a DNA molecule comprising a DNA segment comprising an open reading frame or a portion thereof, wherein the DNA segment has at least 90% nucleic acid sequence identity to a DNA fragment corresponding to a nucleotide sequence between an *EcoRV* site and *EcoRI* site in SEQ ID NO:5.
- 33. (Currently Amended) The expression cassette of claim 32 wherein the DNA segment molecule encodes the a thioesterase domain of pikAIV having at least 90% amino acid sequence identity with SEQ ID NO:37.
- 34. (Currently Amended) The expression cassette of claim 32 wherein the DNA segment molecule encodes the a thioesterase H domain of pikAV having at least 90% amino acid sequence identity with SEQ ID NO:43.

35. (Original) The expression cassette of claim 33 further comprising an acyl carrier protein domain.

- 36. (Currently Amended) The expression cassette of claim 35 further comprising a thioesterase II domain having at least 90% amino acid sequence identity with SEQ ID NO:43.
- 37. (Original) The expression cassette of claim 35 further comprising an acyl transferase domain.
- 38. (Original) The expression cassette of claim 37 further comprising a β -ketoacyl-acyl carrier protein synthase domain.
- 39. (Currently Amended) The expression cassette of any one of claims 32 to 38 wherein the open reading frame encodes DNA molecule comprises a second DNA encoding at least a portion of the N-terminus of PikAI operably linked to the first DNA segment.
- 40. (Original) A host cell transformed with a plasmid comprising the expression cassette of any one of claims 32 to 39.
- 41. (Currently Amended) The host cell of claim 40 which lacks the chromosome of which does not encode a functional thioesterase domain of pikAIV gene cluster having SEO ID NO:37 and the or a functional thioesterase H domain of pikAV gene having SEQ ID NO:43.
- 42. (Withdrawn) A method to alter polyketide chain length, comprising: expressing in a host cell an expression cassette comprising at least a portion of a DNA segment that encodes a module that catalyzes the final condensation of a polyketide so as to yield a polyketide product which is of a different length relative to a polyketide produced by a host cell which does not express the module, wherein the DNA segment that encodes an intact

module encodes two different polypeptides, one of which has a lower molecular weight than the other polypeptide.

- 43. (Withdrawn) The method of claim 42 wherein the intact module is *pikA* module 6.
- 44. (Withdrawn) The method of claim 42 wherein the expression cassette is present on a plasmid.
- 45. (Withdrawn) The method of claim 42 wherein the host cell is a polyketide-producing host cell.
- 46. (Withdrawn) A product produced by the method of claim 42 which is not produced by a host cell which does not express the module.
- 47. (Withdrawn) A method to prepare a polyketide product, comprising: expressing in a host cell an expression cassette comprising a promoter operably linked to a DNA segment comprising a portion of a first polyketide synthase gene so as to yield the product, wherein the expression cassette is present on a plasmid, wherein the chromosome of the host cell comprises at least a portion of a second polyketide synthase gene, and disrupted wherein both portions are operably linked to the native polyketide promoter of one the polyketide genes.
- 48. (Withdrawn) The method of claim 47 wherein the portions are from the same polyketide gene and wherein the portion on the host cell chromosome is different than the portion that is on the plasmid.
- 49. (Withdrawn) The method of claim 48 wherein the portions together comprise the entire gene.
- 50. (Withdrawn) The method of claim 49 wherein the gene is the pikA gene cluster.

- 51. (Withdrawn) A host cell, the genome of which comprises at least a portion of a first polyketide synthase gene, comprising: a plasmid comprising a promoter operably linked to a DNA molecule comprising a DNA segment encoding a portion of a second polyketide synthase gene, wherein both portions are operably linked to the native promoter of one of the genes, and wherein the expression of both portions yields a polyketide.
- 52. (Withdrawn) The host cell of claim 51 wherein the portions are from the same polyketide gene and wherein the portion on the host cell genome is different than the portion that is on the plasmid.
- 53. (Withdrawn) The host cell of claim 52 wherein the portions together comprise the entire gene.
- 54. (Withdrawn) The host cell of claim 53 wherein the gene is the *pikA* gene cluster.
- 55. (Withdrawn) A polyketide produced by the host cell of claim 51.
- 56-60. (Cancelled).
- 61. (Currently Amended) The host cell of claim 40 in which a gene in a *met/pik* biosynthetic gene cluster of the chromosome of the host cell is disrupted or replaced so as to inhibit a beta-ketoacyl-ACP synthase (KS), an acyltransferase (AT), a beta-ketoacyl ACP reductase (KR), or an acyl carrier protein (ACP) having at least 90% sequence identity to SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35 or SEQ ID NO:37, which disruption or replacement alters methymycin, pikromycin, neomethymycin, and/or narbomycin production by the host cell, wherein the gene which is disrupted or replaced has at least about 90% identity to a gene in SEQ ID NO:5 or the complement thereof.

AMENDMENT AND RESPONSE UNDER 37 CFR § 1.116

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Page 8 Dkt: 600.536US1

62. (Currently Amended) The isolated and purified DNA molecule of claim 31 wherein the promoter is a nucleotide sequence in SEQ ID NO:5 between an *EcoRV* position 1982 and an *EcoRI* site in SEQ ID NO:5 position 3133.

63. (New) The expression cassette of claim 31 wherein the promoter is a nucleotide sequence in SEQ ID NO:5 between position 1982 and position 3133.